

Anti-HDAC3 Antibody [SC55-02] - BSA and Azide free

HA750221



Product Type:	Recombinant Rabbit monoclonal IgG, primary antibodies
Species reactivity:	Human, Mouse, Rat
Applications:	WB, IF-Cell, IF-Tissue, IHC-P, IP
Molecular Wt:	Predicted band size: 49 kDa
Clone number:	SC55-02

Description: In the intact cell, DNA closely associates with histones and other nuclear proteins to form chromatin. The remodeling of chromatin is believed to be a critical component of transcriptional regulation and a major source of this remodeling is brought about by the acetylation of nucleosomal histones. Acetylation of lysine residues in the amino-terminal tail domain of histone results in an allosteric change in the nucleosomal conformation and an increased accessibility to transcription factors by DNA. Conversely, the deacetylation of histones is associated with transcriptional silencing. Several mammalian proteins have been identified as nuclear histone acetylases, including GCN5, PCAF (p300/CBP-associated factor), p300/CBP and the TFIID subunit TAF II p250. Mammalian HDAC1 (also designated HD1), HDAC2 (also designated RPD3) and HDAC3, all of which are related to the yeast transcriptional factor Rpd3p, have been identified as histone deacetylases.

Immunogen: Synthetic peptide within C-terminal human HDAC3.

Positive control: HeLa cell lysate, K-562 cell lysate, HEK-293 cell lysate, HT-29 cell lysate, NIH/3T3 cell lysate, C6 cell lysate, COS-1 cell lysate, HeLa, human skin tissue, mouse skin tissue, rat skin tissue, human tonsil tissue, human breast carcinoma tissue.

Subcellular location: Cytoplasm, Nucleus.

Database links: SwissProt: O15379 Human | O88895 Mouse | Q6P6W3 Rat

Recommended Dilutions:

WB	1:1,000-1:2,000
IF-Cell	1:50
IF-Tissue	1:50-1:200
IHC-P	1:200-1:1,000
IP	Use at an assay dependent concentration.

Storage Buffer: PBS (pH7.4).

Storage Instruction: Store at +4℃ after thawing. Aliquot store at -20℃ or -80℃. Avoid repeated freeze / thaw cycles.

Purity: Protein A affinity purified.

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Orders:0086-571-88062880

Technical:0086-571-89986345

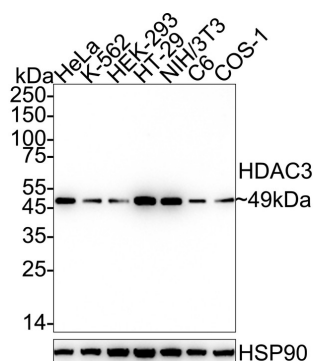
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Applications:WB=Western blot IHC-P=Immunohistochemistry (paraffin) IF-Cell=Immunofluorescence (Cell) IF-Tissue=Immunofluorescence (Tissue) FC=Flow cytometry IP=Immunoprecipitation

Images

Fig1: Western blot analysis of HDAC3 on different lysates with Rabbit anti-HDAC3 antibody (HA750221) at 1/2,000 dilution.



Lane 1: HeLa cell lysate
 Lane 2: K-562 cell lysate
 Lane 3: HEK-293 cell lysate
 Lane 4: HT-29 cell lysate
 Lane 5: NIH/3T3 cell lysate
 Lane 6: C6 cell lysate
 Lane 7: COS-1 cell lysate

Lysates/proteins at 20 µg/Lane.

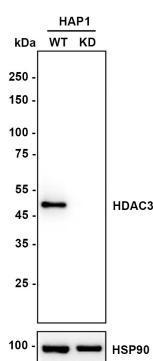
Predicted band size: 49 kDa
 Observed band size: 49 kDa

Exposure time: 3 minutes; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750221) at 1/2,000 dilution was used in 5% NFDM/TBST at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

Fig2: Western blot analysis of HDAC3 on different lysates with Rabbit anti-HDAC3 antibody (HA750221) at 1/5,000 dilution.



Lane 1: HAP1-parental cell lysate
 Lane 2: HAP1-HDAC3 KD cell lysate

Lysates/proteins at 10 µg/Lane.

Predicted band size: 49 kDa
 Observed band size: 49 kDa

Exposure time: 30 seconds; ECL: K1801;

4-20% SDS-PAGE gel.

Proteins were transferred to a PVDF membrane and blocked with 5% NFDM/TBST for 1 hour at room temperature. The primary antibody (HA750221) at 1/5,000 dilution was used in K1803 at 4°C overnight. Goat Anti-Rabbit IgG - HRP Secondary Antibody (HA1001) at 1/50,000 dilution was used for 1 hour at room temperature.

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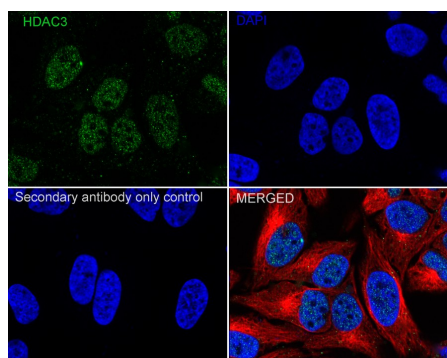
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Fig3: Immunocytochemistry analysis of HeLa cells labeling HDAC3 with Rabbit anti-HDAC3 antibody (HA750221) at 1/50 dilution.



Cells were fixed in 4% paraformaldehyde for 20 minutes at room temperature, permeabilized with 0.1% Triton X-100 in PBS for 5 minutes at room temperature, then blocked with 1% BSA in 10% negative goat serum for 1 hour at room temperature. Cells were then incubated with Rabbit anti-HDAC3 antibody (HA750221) at 1/50 dilution in 1% BSA in PBST overnight at 4 °C. Goat Anti-Rabbit IgG H&L (iFluor™ 488, HA1121) was used as the secondary antibody at 1/1,000 dilution. PBS instead of the primary antibody was used as the secondary antibody only control. Nuclear DNA was labelled in blue with DAPI.

Beta tubulin (M1305-2, red) was stained at 1/100 dilution overnight at +4°C. Goat Anti-Mouse IgG H&L (iFluor™ 594, HA1126) was used as the secondary antibody at 1/1,000 dilution.

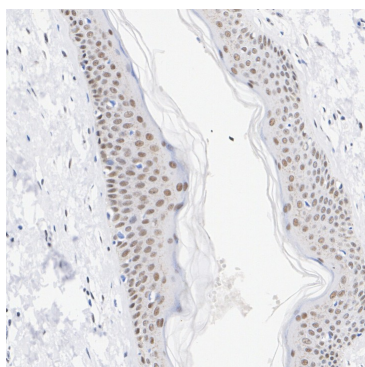


Fig4: Immunohistochemical analysis of paraffin-embedded human skin tissue with Rabbit anti-HDAC3 antibody (HA750221) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA750221) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

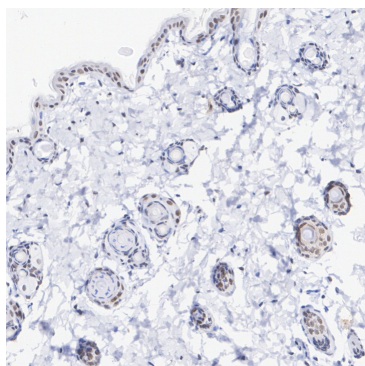


Fig5: Immunohistochemical analysis of paraffin-embedded mouse skin tissue with Rabbit anti-HDAC3 antibody (HA750221) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA750221) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

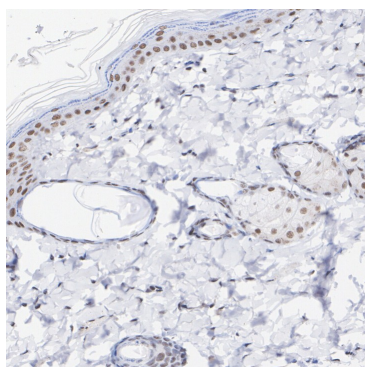


Fig6: Immunohistochemical analysis of paraffin-embedded rat skin tissue with Rabbit anti-HDAC3 antibody (HA750221) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with Tris-EDTA buffer (pH 9.0) for 20 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA750221) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

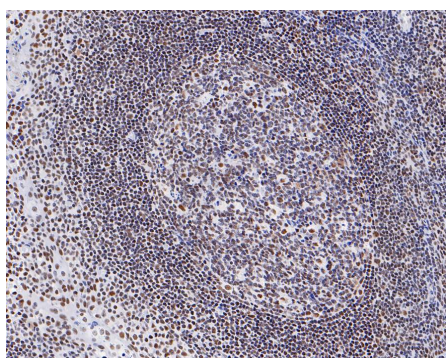


Fig7: Immunohistochemical analysis of paraffin-embedded human tonsil tissue with Rabbit anti-HDAC3 antibody (HA750221) at 1/200 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA750221) at 1/200 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

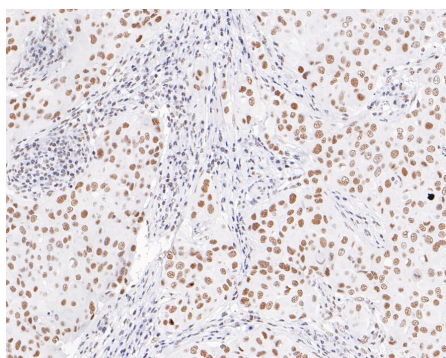


Fig8: Immunohistochemical analysis of paraffin-embedded human breast carcinoma tissue with Rabbit anti-HDAC3 antibody (HA750221) at 1/1,000 dilution.

The section was pre-treated using heat mediated antigen retrieval with sodium citrate buffer (pH 6.0) (high pressure) for 2 minutes. The tissues were blocked in 1% BSA for 20 minutes at room temperature, washed with ddH₂O and PBS, and then probed with the primary antibody (HA750221) at 1/1,000 dilution for 1 hour at room temperature. The detection was performed using an HRP conjugated compact polymer system. DAB was used as the chromogen. Tissues were counterstained with hematoxylin and mounted with DPX.

Note: All products are "FOR RESEARCH USE ONLY AND ARE NOT INTENDED FOR DIAGNOSTIC OR THERAPEUTIC USE".

Background References

1. Jamaladdin S et al. Histone deacetylase (HDAC) 1 and 2 are essential for accurate cell division and the pluripotency of embryonic stem cells. *Proc Natl Acad Sci U S A* 111:9840-5 (2014).
2. Chuang MJ et al. The HDAC inhibitor LBH589 induces ERK-dependent prometaphase arrest in prostate cancer via HDAC6 inactivation and down-regulation. *PLoS One* 8:e73401 (2013).

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